610.5
A595
M497
v. 32
Suppl. 7

ANNALES MEDICINAE EXPERIMENTALIS ET BIOLOGIAE FENNIAE

REDACTORES:

E. MUSTAKALLIO (TURKU) U. UOTILA (HELSINKI) ARMAS VARTIAINEN (HELSINKI)

ALVAR WILSKA (HELSINKI) A. I. VIRTANEN (HELSINKI)

EDITOR

K. O. RENKONEN

REDIGENDOS CURAVIT

ODD WAGER

PYRIDINENUCLEOTIDES (COENZYME I AND II) OF BLOOD IN NORMAL SUBJECTS AND IN VARIOUS DISEASES, ESPECIALLY TUBERCULOSIS

BY

WILLIAM KERPPOLA and JORMA PÄTIÄLÄ

VOL. 32 1954 SUPPLEMENTUM 7

MERCATORIN KIRJAPAINO
HELSINKI, FINLAND

UNIVERSITY OF MICHIGAN
MEDICAL LIBRARY

FROM THE FIRST MEDICAL DEPARTMENT, UNIVERSITY
OF HELSINKI, FINLAND

PYRIDINENUCLEOTIDES (COENZYME I AND II) OF BLOOD IN NORMAL SUBJECTS AND IN VARIOUS DISEASES, ESPECIALLY TUBERCULOSIS

BY

WILLIAM KERPPOLA and JORMA PÄTIÄLÄ

HELSINKI 1954

610.5
A595
M497
v. 32
suppl. 7

HELSINKI 1954
MERCATORIN KIRJAPAINO

Medical Lib.
exchange
dept
12-20-54

PYRIDINENUCLEOTIDES (COENZYME I AND II) OF BLOOD IN NORMAL SUBJECTS AND IN VARIOUS DISEASES, ESPECIALLY TUBERCULOSIS[1]

by

WILLIAM KERPPOLA AND JORMA PÄTIÄLÄ

After Elvehjelm and others (1938) had explained the vitamin action of nicotinic acid, both this and its amide have been frequently studied. It has been found out that nicotinamide or niacine is an essential part of the prosthetic group of certain enzymes. Three of the coenzymes are known: diphosphopyridinenucleotide (DPN) or codehydrogenase I or coenzyme I, triphosphopyridinenucleotide (TPN) or codehydrogenase II or coenzyme II, and coenzyme III, the existense of which has been recently discovered. The term codehydrase was formerly used instead of coenzyme.

The above-mentioned coenzymes may occur in oxidized and reduced form. When coenzyme is reduced, the substratum will be oxidized, i.e. it usually undergoes dehydrogenation. In order to make it possible for the prosthetic group to become active again, it has to be returned into oxidized form. The DPN- (and TPN-) cytochrome c reductase is able to achieve this; its prosthetic group contains flavinadeninedinucleotide. In its reduced form the enzyme is able directly to react upon oxygen, but cytochrome c oxidizes it more rapidly. It is assumed that reduced cytochrome c will be oxidized by means of cytochromeoxidase, which in its reduced form, easily oxidizes by means of molecular oxygen. Enzymes, whose prosthetic group is made up of DPN or TPN, are a number, so far, more than ten. Among these we may mention glucose-6-phosphate dehydrogenase, lactic acid dehydrogenase

[1] An Award from Sigrid Juselius Foundation made this work possible.

and alcohol dehydrogenase, and many of the dehydrogenases in Krebs's cycle. These enzymes play an important role in the metabolism of sugar.

Coenzymes with a nicotinamide content occur in almost all animal tissues, in which their amount varies, however. In blood they are present in the red cells, in which their content is comparatively fixed, even in serious deficiency diseases. But in liver, muscles, heart and brain their amount is reduced by continued defiency.

Levitas and others obtained with a fluorometric method the following values for pyridinenucleotides from a normal series of 20 persons (1947): in whole blood between 28 γ and 44 γ per ml, on an average 36 ± 5 γ per ml, and in red cells between 62 γ and 89 γ per ml, on an average $77 + 8$ γ per ml. Vilter and others (1939) arrived at roughly similar values by emplying the biological method on a series of 50 healty persons, and Rossini (1949) in 12 normal subjects.

As regards the determination of the pyridinenucleotides in question in various diseases, there are only few investigations on this subject to be found in literature. Determination has been performed in all of them by the biological method, by means of bacillus influenzae and parainfluenzae. Kohn (1938) and Vilter together with others (1939) found that the coenzymes I and II are decreased in the blood of patients ill with clinical pellagra. In these studies it was observed that the degree of the deficiency of coenzymes in question in whole blood and in red cells did not parallel the severity of any one of the pellagrous symptoms, but it did parallel in general the clinical state of the patient. Vilter and others (1939) carried on their studies in pyridinenucleotides in three severe diabetic acidosis patients. They found out that the blood of these cases, when inoculated with loop transfers from a 12-hour culture of B. influenzae, supported growth of this organism in dilutions of 1/2000 or less. In contrast, the blood of the control series invariably supported growth in dilutions of 1/8000 or more. Sydenstricher and others (1939) have shown clinically in diabetic patient under insulin therapy that a very delicate balance exists in certain diabetic persons who have subsisted for long periods of time on inadequate and unbalanced diets and who have frequent bouts of active pellagra.

Vilter and others (1940) finally studied 20 patients with typical pneumocóccal pneumonia of less than five days' duration and a

control group of 50 normal subjects. They discovered a significant decrease in the concentration of coenzymes I and II in the blood of 17 patients with acute lobar pneumonia, as determined by the growth stimulation for the influenza bacillus. In this connection we do well to remember that, as is commonly known, pneumonia may precipitate an attack of acute pellagra in undernourished persons (Spies 1937).

We have found in literature only one treatise on the occurence of pyridinenucleotides in the blood of tuberculous patients, viz. that by Rossini, from the year 1949. By means of the biological method he determined the codehydrase content of the blood in 12 normal subjects and in 17 patients suffering from pulmonary tuberculosis. Only four tuberculous patients had the normal amount of 60, another four a reduced amount of 30, and seven a considerably reduced amount of 15 per ml., while two cachetic patients showed a still lower value of only 8 per ml. The codehydrase amount as a rule seems to have been lower in those patients whose disease was more severe and whose general condition was worse than in the rest.

On the other hand, several investigations have shown that tuberculosis is accompanied with a disturbance of P.P. vitamin. Low values for nicotinic acid in blood have been determined among others by Oliva and Magrini (1943) in peritoneal tuberculosis and in tuberculous patients affected with diarrhoea, whereas in pulmonary tuberculosis, even in malignant cases, normal values often occurred. In the same way, in Rossinins above-mentioned series only two out of 17 tuberculous patients (tub.pulm.) showed a clearly decreased value. But on the other hand, Bezancon (1943) found mostly decreased values also in tuberculosis of the lung.

MATERIAL AND METHODS OF INVESTIGATION

The patients ill with pulmonary tuberculosis that are included in the present series have been under treatment in the University Department of the Tuberculosis Hospital, Helsinki. The psychiatric cases were from the Psychiatric Department of the University of Helsinki.[1] All the other cases have been treated at the First

[1] The authors wish to record their gratitude to Professor Martti Kaila, Head of the Department, for the kindness of having placed the present material at their disposal.

Medical Department of the University of Helsinki, where the patients as well as those in the Psychiatric Department come from all parts of the country and from every class of society.

The material is divided between the different disease groups in the following manner.

Tuberculosis		92	cases
Pulmonary tuberculosis	79		
Other tuberculosis	6		
Exudative pleurisy	7		
Lung diseases		17	»
Bronchial asthma	7		
Bronchopneumonia	4		
Other lung diseases	6		
Neurocirculatory asthenia		25	»
Schizophrenia		17	»
Endocrine and metabolic diseases		27	»
Diabetes mellitus	6		
Struma non toxica	7		
Thyreotoxicosis	3		
Other endocrine diseases	5		
Adiposity	6		
Diseases of digestive system		30	»
Achylia gastrica	7		
Duodenal and gastric ulcer	12		
Hepatic diseases	5		
Diseases of the intestines	6		
Leucemia and other blood diseases		12	»
Rheumatic diseases		10	»
Heart and circulatory diseases		12	»
Renal diseases		3	»
Carcinoma and other malignant tumours		8	»
		Total 253	cases

Besides, the material includes 41 healthy persons, mainly medical students and nurses. Our material is thus made up of 294 persons all told.

The patients in the three hospitals in questions were given the ordinary hospital food, with the exception of the ulcer patients,

who were on diet. They were not given any drugs containing vitamin B. The investigation of blood was performed in the morning before the patient had taken any kind of food.

The method used for the determination of the total pyridinenucleotides in blood is a fluorometric method by Nora Levitas et al (1947).

The alcali acetone condensation reaction for N_1-methylnicotinamide yields with both pyridinenucleotides a highly fluorescent product whose fluorescence intensity is about twice as high, per equivalent, as that of the N_1-methylnicotinamide derivate. It can be used for the quantitative determination of the total coenzymes in amounts as small as 1 γ, corresponding to 0.05 ml. of whole blood or 0.02 ml. of red cells. The oxalated blood is deproteinized with trichloracetic acid, the condensation reaction is carried out on the filtrate, and the fluorescence read in the fluorometer within 10 minutes after filtration.

An internal standard of N_1-methylnicotinamide is employed and the results are calculated in terms of either diphosphopyridinenucleotide or nicotinic acid per ml. of erythrocytes, on the basis of the recovery value and of the simultaneously determined hematocrit. It is emphatically suggested that whole blood of nicotinic acid or of the pyridinenucleotides are both useless and misleading, unless the corresponding hematocrit readings are given.

REAGENTS AND APPARATUS

— 25 per cent trichloracetic acid.
— 6-n NaOH, prepared daily by dilution of the carbonate-free saturated solution (about 19-n).
— 6-n HCl, any C.P. grade of concentrated hydrochloric acid diluted 1: 1 with water.
— redistilled acetone, rendered free of fluorescent substances by distillation over $KMnO_4$.
— 20 per cent KH_2PO_4 solution.
— the standard solution of N_1-methylnicotinamide containing 1 per ml.
— the standard quinine sulfate solution containing 0.3 per ml. in 0.1—n H_2SO_4.
— fluorometer.
— cuvette tubes graduated at 10 ml.

PROCEDURE

Since it has been amply demonstrated that practically all of the nicotinic acid derivates are confined to the red blood cells, the determination may be carried out on the whole blood without any loss of accuracy if hematocrit determinations are made.

2.5 ml. of venous blood was drawn into a bottle containing a previously dried mixture of 3 mg. of ammonium oxalate and 2 mg of potassium oxalate. This amount is sufficient for 2.5 ml. of blood without affecting the cell volume. The blood should be laked and precipitated simultaneously and as soon as possible because the coenzymes are rapidly destroyed upon hemolysis. If hemolysis has not occurred, the blood can stand for as long as 24 hours without loss of diphosphopyridinenucleotide.

The determination of hematocrit is carried out with 0.1 ml of the oxalated blood.

Into a large testtube (20×180 mm) or a 50 ml round bottomed centrifuge cup, containing 2 ml. of 25 per cent trichloracetic acid and 6 ml. of water, are added dropwise and with constant shaking 2 ml. of whole blood. The contents are well mixed by rapid inversion for 1 minute. The solution is allowed to stand for several minutes and is then centrifuged 5 to 10 min. at 3000 r.p.m. and filtered through a 7 cm N:o 30 Whatman paper. The clear filtrate is stable and will not deteriorate for several days if kept in the refrigerator. This filtrate provides more than enough material for duplicate analyses.

Into the bottom of each of three graduated test tubes is measured 0.5 ml. of blood filtrate. To tube 1 is added 0.5 ml. of water, to tube 2, the blank, 1 ml. of water, to tube 3, the recovery 0.50 of N_1-methylnicotinamide contained in 0.5 ml. of the working standard solution.

To tubes 1 and 3, 0.5 ml. of acetone is added and the contents are mixed. To each of tubes there is added 0.20 ml. of 6-n NaOH directly into the solution, touching off the last drop near the surface of the solution and mixing the contents of each tube immediately after the addition of the alkali. The tubes are allowed to stand at room temperature for 5 min. To each tube 0.3 ml. of 6-n HCl is added, mixing each tube at once after the addition. The tubes are immersed into a boiling water bath for 2 minutes, 1 ml. of 20 per cent KH_2PO_4 solution is added, and the contents diluted with water to the 10 ml mark and mixed. The fluorescence reaches a maximum immediately and remains stable in the daylight or in the dark for at least 3 days. Therefore it may measured at any convenient time.

The intensity of the fluorescence of the compound obtained from blood filtrates by this method follows the same curves with respect to temperature and pH as those which are given by Huff and Perlzweig for N_1-methylnicotinamide.

Diphosphopyridinenucleotide of per cent purity, gives a recovery value with blood filtrate of 15 divisions per microgram, while N_1-methylnicotinamide added to an aliquot of the same filtrate yields a value of 38 divisions per microgram in the Coleman model 12 fluorometer. This proportionality factor, 15/38 = 0.4, remains constant, so that if recovery values other than 38 divisions per microgram of N_1-methylnicotinamide are encountered a correct diphosphopyridinenucleotide value be calculated from the above factor. However, it must be realized that this is a recovery value and is not the same as that obtained with the standard solution alone.

Example of Calculation. — Hematocrit 42%. Dilution, 2 ml. whole

blood to 10 ml. 0.5 ml. filtraten (= 0.1 ml. blood) taken for analysis, also 0.5 ml. filtrate + 0.5 γ N_1-methylnicotinamide for recovery value.

Readings.

Blood filtrate 58 divisions
Blank 12 »
Recovery 78 »

58—12 = 46 divisions for 0.1 ml whole blood
78—58 = 20 divisions for 0.5 $\ddot{u}$ N_1-methylnicotinamide
= 40 divisions for 1 $\ddot{u}$ »

$40 \times 0.4 = 16$ divisions for 1 γ diphosphopyridinenucleotide

$\frac{46}{16} \times \frac{1}{0,1} = 29$ γ diphosphopyridinenucleotide per 1 ml. whole blood

$\frac{29}{42} \times 100 = 68$ γ diphosphopyridinenucleotide per 1 ml erythrocytes

All fluorescent determinations are made by using the Beckman model »DU» Quartzspectrophotometer.

In all tests a double determination was performed. Without exception, the results of each determination were well in accord with each other, which proves the reliability of the method employed.

Excepting cases, of which special mention is made, no cases of more or less marked anaemia were used for this investigation. In each case the hematocrit of blood was determined.

The mathematical treatment of the results was performed by a mathematician.

Our normal series was made up to of 41 persons in age of 20 to 36 years, of which 19 were men and 22 women. All of them felt perfectly well, neither were any symptoms of illness found in them means of a clinical examination. These persons had not been on any diet and had not taken any drugs containing vitamin B. The results are to be seen in Table I.

The amount of pyridinenucleotides in whole blood varied from 20.0 to 37.1 γ per ml., the average being 28.1 ± 0.69 γ per ml., and in red cells from 51.7 to 90.0 γ per ml., on an average 67.1 ± 1.49 γ per ml. The value for hematocrit varied between 34 and 55, average 41.9.

The tuberculosis series was made up of 78 patients ill with pulmonary tuberculosis, 6 cases of extrapulmonary tuberculosis, and 7 cases of exudative pleurisy. The averages — 24.0 ± 0.34 γ per ml. in whole blood and 54.0 ± 0.60 γ per ml. in red cells — show that the figure for pyridinenucleotides displays a clear decrease as compared with the corresponding figures in the normal series. In terms of statistical mathematics the difference is almost significant in whole blood and highly significant in red cells.

TABLE I

PYRIDINENUCLEOTIDES OF BLOOD IN NORMAL SUBJECTS

No.	Age	Sex	Hematocrit	Pyridinenucleotides	
				In Whole Blood γ per ml.	In Red Cells γ per ml.
1	29	f	40	32.4	79.2
2	25	»	38	33.3	90.0
3	22	m	45	31.8	70.6
4	21	»	44	30.3	68.8
5	22	»	48	30.4	63.0
6	20	»	47	37.1	78.9
7	23	»	45	32.1	71.3
8	21	»	45	35.8	79.6
9	22	»	45	28.8	63.8
10	22	»	36	23.1	64.2
11	21	f	34	24.5	72.9
12	21	»	41	31.6	76.8
13	33	»	39	33.2	85.1
14	27	»	38	26.1	68.7
15	33	m	43	31.3	72.8
16	21	f	43	23.4	54.4
17	22	»	42	26.7	63.6
18	23	»	35	24.8	70.9
19	35	»	40	22.3	55.8
20	20	»	43	25.7	59.8
21	20	»	39	20.8	51.7
22	26	»	42	22.2	52.8
23	30	»	40	21.8	54.5
24	36	»	45	26.5	59.0
25	22	»	41	23.8	58.0
26	24	»	40	21.5	53.8
27	22	m	45	26.9	59.8
28	24	f	36	20.0	57.0
29	23	m	41	26.3	64.2
30	22	»	43	28.6	66.6
31	22	f	35	23.9	68.5
32	21	m	40	26.7	66.7
33	30	»	43	24.4	56.8
34	28	»	40	31.7	79.4
35	22	f	43	35.2	82.0
36	22	m	53	32.9	62.2
37	30	f	42	30.8	73.4
38	23	»	41	30.7	75.0
39	22	m	48	32.2	67.1
40	21	»	41	29.2	63.5
41	30	»	40	28.3	70.8
			Mean 41.9	Mean 28.1 $\pm$ 0.69	Mean 67.1 $\pm$ 1.49

TABLE IIa

PYRIDINENUCLEOTIDES OF BLOOD IN TUBERCULOSIS

No	Age	Sex	Hematocrit	Pyridinenucleotides		Diagnosis			
				In Whole Blood γ per ml.	In Red Cells γ per ml.				
1	35	m	41	24.1	58.8	Tub.	pulm.	Ia.	
2	34	»	40	24.0	60.0	»	»	»	
3	25	»	42	23.1	55.0	»	»	»	
4	20	»	47	22.7	48.3	»	»	»	
5	31	»	54	27.8	51.5	»	»	»	
6	30	»	47	27.0	55.3	»	»	»	
7	41	»	35	19.0	54.3	Tub.	pulm.	Ib.	
8	49	»	39	21.2	51.3	Tub.	pulm.	Ic.	
9	33	»	45	23.4	52.0	»	»	»	
10	55	»	40	23.5	58.8	Tub.	pulm.	IIa.	
11	35	»	47	20.7	44.6	»	»	»	
12	41	»	43	25.4	59.0	»	»	»	
13	60	»	54	27.1	50.2	»	»	»	
14	58	»	33	19.8	60.0	»	»	»	
15	41	»	53	31.3	59.1	Tub.	pulm.	IIb.	
16	60	»	41	22.5	54.9	»	»	»	
17	43	»	45	28.8	62.9	»	»	»	
18	30	»	41	23.6	57.6	»	»	»	
19	31	»	39	24.5	62.8	»	»	»	
20	36	»	46	23.4	50.9	Tub.	pulm.	IIc.	
21	38	»	44	22.4	50.9	»	»	»	
22	32	»	54	30.0	55.6	»	»	»	
23	37	»	46	27.8	60.4	»	»	»	
24	71	»	48	25.6	53.3	Tub.	pulm.	IIIa.	cav.
25	23	»	47	25.9	55.1	»	»	»	
26	46	»	48	24.5	51.0	»	»	»	cav.
27	30	»	52	25.9	49.8	»	»	»	cav.
28	29	»	46	20.1	40.2	»	»	»	cav.
29	51	»	49	25.3	51.6	»	»	»	
30	58	»	50	28.4	56.8	»	»	»	
31	49	»	42	20.1	47.9	»	»	»	cav.
32	20	»	48	25.2	52.5	»	»	»	
33	43	»	45	23.9	53.1	»	»	»	cav.
34	62	»	34	20.2	59.4	»	»	»	
35	71	»	42	21.7	51.7	»	»	»	
36	22	»	47	24.7	52.6	»	»	»	cav.
37	61	»	47	27.1	57.7	»	»	»	
38	72	»	54	30.1	53.9	»	»	»	
39	33	»	45	24.0	53.2	»	»	»	
40	57	»	39	21.4	54.9	»	»	»	
41	74	»	51	24.2	47.5	»	»	»	cav.
42	60	»	47	23.9	50.9	»	»	»	cav.
43	52	»	39	20.6	52.8	»	»	»	
44	51	»	45	18.7	41.6	»	»	»	
45	55	»	43	20.3	47.2	»	»	»	
46	44	»	35	17.6	50.3	»	»	»	
47	31	»	40	23.1	57.8	»	»	»	cav.
48	26	»	40	21.4	53.5	»	»	»	
49	42	»	48	26.0	54.2	»	»	»	

No	Age	Sex	Hematocrit	Pyridinenucleotides		Diagnosis
				In Whole Blood γ per ml.	In Red Cells γ per ml.	
50	38	m	52	28.5	54.8	Tub. pulm. IIIa
51	60	»	45	24.4	54.2	» » »
52	55	»	46	18.3	39.8	» » » cav.
53	49	»	42	20.1	47.9	» » » cav.
54	62	»	49	22.9	46.7	» » » cav.
55	28	»	47	22.1	47.0	» » »
56	57	»	51	21.9	42.9	» » » cav.
57	43	»	50	26.4	52.8	» » »
58	32	»	47	23.1	49.1	» » »
59	40	»	50	23.1	46.2	» » »
60	50	»	48	22.2	46.3	» » »
61	72	»	45	24.9	55.5	» » »
62	56	»	42	21.6	51.4	» » »
63	46	»	48	28.8	59.8	» » »
64	27	»	47	26.8	57.2	» » »
65	59	»	50	25.4	50.8	» » » cav.
66	38	»	46	25.4	55.2	» » »
67	40	»	41	27.6	67.4	» » »
68	32	»	48	26.3	54.8	» » »
69	34	»	41	20.3	49.5	» » »
70	54	»	41	21.4	52.2	» » »
71	30	»	40	21.9	54.8	» » » cav.
72	35	»	39	23.4	60.0	Tub. pulm. IIIb.
73	53	»	50	31.5	63.0	» » »
74	28	»	47	27.3	58.1	» » »
75	39	»	47	30.8	65.5	» » »
76	29	»	44	26.7	60.7	» » »
77	40	»	43	22.5	52.4	Tub. pulm. IIIc.
78	31	»	41	22.3	54.4	» » »
79	33	»	45	25.6	56.9	» » »
80	41	»	48	24.9	51.9	Pleuritis exsudativa
81	54	»	54	24.6	55.8	» »
82	30	»	41	20.7	50.5	» »
83	37	»	37	19.8	53.6	» »
84	65	»	39	17.8	45.7	» »
85	41	»	42	25.6	61.0	» »
86	16	»	49	21.9	56.3	» »
87	22	f	41	24.9	60.7	Lymphomata tub. colli
88	44	»	42	22.1	52.6	Tub. renis
89	46	»	51	31.5	61.8	» »
90	47	»	49	27.0	57.0	Tub. vertebrae
91	21	»	37	24.6	66.5	Meningitis tub.
92	35	»	35	21.5	61.5	Lupus vulgaris
			Mean 45.0 $\pm$ 0.53	Mean 24.0 $\pm$ 0.34	Mean 54.0 $\pm$ 0.60	

Pulmonary tuberculosis: Without exception the diagnosis was verified bacteriologically. Determination of pyridinenucleotides was performed in the beginning of the treatment, before actual therapeutic measures were taken. The clinical classification and the diagnosis were worked out according to a schedule suggested by Forsman and Larmola (1944), which for the present has been generally adopted in Finland. In this classification both the stage and the nature of the disease are observed os follows. Group a. Rapidly progressing forms of the disease. Group b. Slowly progressing and relatively inactive forms of the disease. Group c. Mixed forms. The classification according to the nature of the disease is based on the following criteria. 1. Anamnesis and decursus morbi during the period of observation. 2. Finding by means of X:rays. 3. Temperature during the period of observation. 4. Sedimentation rate. How the present series was divided on the basis of this classification is to be seen from the Table II b.

The Table shows that the exudative a-type was the most frequent one, amounting to as many as 58 cases, whereas there were only 11 cases of the benign productive b-type, the number of the cases representing the intermediate type c being 9. The averages of pyridinenucleotides in the different groups were as follows.

TABLE IIb

Nature of the Disease	Number of Cases	Hematocrit	Pyridinenucleotides	
			Whole Blood γ per ml.	Red Cells γ per ml.
a	58	45.4 $\pm$ 0.64	23.7 $\pm$ 0.28	52.3 $\pm$ 0.70
b	11	43.7 $\pm$ 1.69	26.3 $\pm$ 1.24	60.0 $\pm$ 1.07
c	9	44.7 $\pm$ 1.40	24.3 $\pm$ 0.98	53.9 $\pm$ 1.09

The gratest decrease of pyridinenucleotides both in whole blood and in red cells was to be found in type a of the disease, the smallest in type b. Low values occurred especially in the 16 patients representing type a of disease who had cavities (Table II a). Their average of pyridinenucleotides was 22.9 $\pm$ 0.58 γ per ml. in whole blood and 49.2 $\pm$ 1.26 γ per ml. in red cells. Hematocrit was 46.5 $\pm$ 1.0. It was in this group that the lowest pyridinenucleotide value in the entire material. viz. 18.3 γ per ml. in whole blood and 39.8 γ per ml. in red

cells was met with. (case 52). The patient in question was in a very poor condition, with high temperature, in fact, he died one month after the investigation.

The decrease of pyridinenucleotides in tuberculous patients accordingly seems to a considerable degree to depend on the type of disease in question. On the other hand, the stage of the disease does not appear to be of the same significance as will be seen from the following table II c.

TABLE IIc

Nature of the Disease	Number of Cases	Hematocrit	Pyridinenucleotides	
			Whole Blood γ per ml.	Red Cells γ per ml.
I stage	9	43.3 ± 1.87	23.6 ± 0.90	54.1 ± 1.25
II »	14	44.8 ± 1.62	25.6 ± 0.93	56.4 ± 1.43
III »	55	45.4 ± 0.59	24.0 ± 0.43	52.8 ± 0.77

Exudative pleurisy: The material consisted of 7 cases of tuberculous pleurisy, in all of which the disease was still in a comparatively acute phase. Pyridinenucleotides showed decrease, and also their averages, 22.9 ± 0.58 γ per ml. in whole blood and 53.6 ± 1.84 γ per ml. in red cells were clearly decreased. These figures come very near to the corresponding averages representing type a of pulmonary tuberculosis.

Other tuberculosis: The material comprised the following cases of extrapulmonary tuberculosis: 1 lymphomata tub. colli, 2 tub. renis, 1 tub. vertebrae, 1 meningitis tuberculosa and 1 lupus vulgaris. Only one of the two cases of renal tuberculosis shows a clear decrease of pyridinenucleotides. The patient in question had temperature and his general condition had deteriorated at the time of the examination, whereas the other patients belonging to this group were free from fever and in a relatively good condition. As regards the case of meningitis tuberculosa, the patient had already been under treatment for a few months prior to the determination of pyridinenucleotides, and had been given, among other drugs, streptomycin all the time. So the patient was at this point of time free from fever and his general condition rather satisfactory. The number of cells in liquor was 110. The fact that he complained of headache was the only subjective symptom.

TABLE III

PYRIDINENUCLEOTIDES OF BLOOD IN SOME LUNG DISEASAS

No	Age	Sex	Hematocrit	Pyridinenucleotides		Diagnosis
				In Whole Blood γ per ml.	In Red Cells γ per ml.	
1	46	f	39	37.3	80.3	Asthma bronchiale
2	48	»	40	26.2	65.5	» »
3	49	m	48	29.1	60.5	» »
4	17	f	46	24.7	53.7	» »
5	46	»	49	31.2	64.5	» »
6	49	m	46	33.8	75.2	» »
7	46	f	41	23.7	58.8	» »
			Mean 44.1 $\pm$ 1.54	Mean 28.6 $\pm$ 1.39	Mean 65.4 $\pm$ 3.54	
8	61	m	42	23	54.8	Bronchopneumonia
9	18	»	45	24	53.4	»
10	20	»	45	25.2	56.1	»
11	37	»	44	23.8	54.1	»
			Mean 44 $\pm$ 0.71	Mean 24.0 $\pm$ 0.46	Mean 54.7 $\pm$ 0.58	
12	51	m	42	22.2	52.8	Bronchiectasiae
13	51	»	46	30.7	66.7	»
14	53	»	40	25.3	63.3	Bronchitis acuta
15	65	»	40	23.8	59.5	Emphysema pulm.
16	50	»	37	25.6	69.2	» »
17	48	»	43	31.9	74.1	» »

Bronchial asthma: The average figure for pyridinenucleotides is normal in whole blood as well as in red cells. As can be seen from Table III, there are only instances of averages below normal. The patient in case 4 had severe attacks of asthma at the time of the present examination; the percentage of eosinophiles in blood was 22, and besides he suffered from sinusitis maxillaris.

Bronchopneumonia: This group comprised 4 patients ill with bronchopneumonia, whose disease, exepting case 10, was in a comparatively acute phase. As can be gathered from Table III, the figure for pyridinenucleotides shows decrease in all cases, and also the average is below the corresponding normal figure. However, the number of the cases is very small.

Other lung diseases: This group was made up of two bronchiectasies, one bronchitis acuta, and three emphysema pulmonum. In individual cases only once a somewhat decreased value was obtained.

TABLE IV

PYRIDINENUCLEOTIDES OF BLOOD IN NEUROCIRCULATORY ASTHENIA

No	Age	Sex	Hematocrit	Pyridinenucleotides	
				In Whole Blood γ per ml.	In Red Cells γ per ml.
1	59	f	41	23.2	56.4
2	33	m	41	32.8	80.0
3	24	f	45	32.3	71.8
4	17	»	43	24.8	57.7
5	22	»	47	30.6	65.1
6	32	»	43	21.8	50.7
7	25	m	50	23.4	46.8
8	42	»	52	25.8	49.6
9	27	f	38	17.4	45.8
10	36	»	35	21.4	61.2
11	40	»	41	22.8	55.6
12	42	m	42	27.0	67.5
13	34	»	39	26.0	66.8
14	30	f	38	19.2	50.5
15	57	»	43	23.2	54.0
16	44	m	48	30.3	63.2
17	37	»	54	32.5	60.0
18	28	f	40	22.0	55.0
19	42	m	46	27.5	59.8
20	41	»	42	23.2	57.7
21	38	»	41	26.2	64.0
22	42	»	43	24.2	56.3
23	32	»	45	26.3	58.5
24	46	»	41	21.3	50.6
25	22	»	52	29.9	56.7
			Mean 43.4 ± 0.94	Mean 25.4 ± 0.87	Mean 59.0 ± 1.69

Neurocirculatory asthenia: The material consists of 25 cases diagnosed as neurocirculatory asthenia. The diagnosis, established in the First Medical Department, was mainly based on the following typical history. These patients complain of many kinds of troubles, for which no explanation can be found in an objective examination of the different organs. They are often young or middle-aged persons of athletic constitution. They easily manifest palpitations and extrasystoles from strain and psychis causes, often without any known reason, e.g. at night during rest. They tolerate physical exertion badly and get easily tired. They are often short of

breath, even at rest, yawn and sigh. Vertigo and headache are common. Many of these persons perspire easily, especially from exertion. Their sleep is broken, they have strange sensations in the extremities (pricking, stiffness, trembling, »cramps»). Physical and mental strain aggravate these troubles. Most of them also complain of vague abdominal pains, often found to be due to excessive fermentation of carbohydrates in the intestines, which is usually objectively confirmed by Schmidt-Strassburger's fermentation test. Inability to stand the heat in the Finnish bath (»Sauna»), which aggravates their condition, is a noteworthy sign. They react sensitively to tobacco, coffee, and alcohol. In alcohol tolerance tests their capacity to eliminate alcohol from the blood has been found to be lower than that of healthy individuals (Jokipii 1951).

As can be seen from the Table IV, the amount of pyridinenucleotides both in whole blood and in red cells often shows clear decrease, and correspondingly, the average figures, 25.4 ± 0.87 γ per ml. and 59.0 ± 1.69 γ per ml. respectively, are clearly lower than the cor-

TABLE V

PYRIDINENUCLEOTIDES OF BLOOD IN SCHIZOPHRENIA

No	Age	Sex	Hematocrit	Pyridinenucleotides	
				In Whole Blood γ per ml.	In Red Cells γ per ml.
1	33	m	42	21.1	50.2
2	34	»	40	25.7	64.3
3	30	»	52	30.0	57.7
4	43	»	49	24.6	50.4
5	42	»	42	23.2	55.3
6	40	f	42	27.0	64.3
7	36	»	42	19.2	46.7
8	48	»	41	26.8	65.3
9	50	m	42	24.2	57.7
10	28	»	43	23.5	54.7
11	29	»	46	27.6	60.0
12	34	f	42	21.8	51.8
13	28	»	43	23.8	55.5
14	24	»	38	20.2	53.2
15	25	»	45	24.9	55.5
16	16	m	48	25.8	53.8
17	50	»	48	28.4	59.2
			Mean 43.9 ± 0.88	Mean 24.6 ± 0.72	Mean 56.2 ± 1.28

TABLE VI

PYRIDINENUCLEOTIDES OF BLOOD IN ENDOCRINE DISEASES

No	Age	Sex	Hematocrit	Pridinenuceolides		Diagnosis
				In Whole Blood γ per ml.	In Red Cells γ per ml.	
1	61	f	47	36.3	77.2	Diabetes mellitus
2	52	m	46	24.7	53.7	» »
3	61	f	44	31.1	70.7	» »
4	58	m	41	29.9	79.0	» »
5	22	f	42	22.0	52.4	» »
6	28	m	44	25.7	58.4	» »
			Mean 43.6 $\pm$ 2.59	Mean 28.3 $\pm$ 2.11	Mean 65.2 $\pm$ 4.86	
7	18	f	45	30.4	67.6	Struma non toxica
8	42	»	41	23.6	57.5	» » »
9	16	»	40	21.2	52.9	» » »
10	43	»	44	23.4	53.3	» » »
11	29	»	40	22.2	60.5	» » »
12	65	»	40	24.2	60.5	» » »
13	40	»	45	25.7	57.0	» » »
14	32	m	50	27.5	55.0	Thyreotoxicosis
15	27	»	41	26.3	66.6	»
16	25	»	36	22.4	62.2	»
17	66	f	44	32.6	74.1	Adiposity
18	57	»	44	22.2	50.5	»
19	61	m	40	27.6	68.9	»
20	48	»	45	26.1	58.0	»
21	48	f	37	22.2	59.5	»
22	61	m	45	36.6	81.2	»
			Mean 42.5 $\pm$ 1.34	Mean 27.9 $\pm$ 2.36	Mean 65.4 $\pm$ 4.65	
23	18	m	49	36.3	74	Cushings Syndrome
24	34	»	42	27.6	65.8	» »
25	35	f	33	26.4	80.0	Acromegalia
26	31	m	50	28.0	56.0	Hyperinsulismus
27	50	f	40	36.3	90.8	Climacterium

responding figures in the normal series. Statistical treatment too shows the difference between the averages to be significant, as regards red cells rather much so at that. As for the individual cases it is worth notice that e.g. in red cells the amount of pyridinenucleotides falls three times below 50 γ per ml., while higher values of 70 γ per ml. and more are to be found in only two cases.

Schizophrenia: This group was made up of 17 cases of schizophrenia. No symptoms of inflammatory or other diseases were found in the patients in a clinical examination. The Table V shows that the total figure for pyridinenucleotides in this group is a clearly decreased one. In comparison with normal series the difference is almost significant, to use the term of statistical mathematics.

Diabetes mellitus: The material comprised 6 patients ill with diabetes mellitus. With the exception of one, all of them were treated with insulin. It can be seen from the Table VI that the average of pyridinenucleotides is normal in whole blood as well as in red cells. Cases 2 and 5, which show a value clearly below the average, were subfebrile at the time of the examination, due to acute infection.

Struma non toxica and *thyreotoxicosis:* This group consisted of 7 cases of clinically unmistakable non-toxic struma and 3 cases of thyreotoxicosis, where the diagnosis was established on the basis of a typical clinical picture, and of increased iodine content in blood and an elevated basal metabolic rate. All the patients had a diffusely enlarged thyroid gland. In case 9, where the lowest rate of pyridinenucleotides was found, the patient was also suffering from cholecystitis.

Adiposity: The material includes 6 cases of adiposity, three of which had hypertension. The average of pyridinenucleotides is normal; only case 18 shows a value clearly below the normal, especially in red cells: 50.5 γ per ml. The patient in question had temperature at the time of the examination and showed symptoms of apoplexy.

Some other endocrine diseases: There were two cases of Cushing's syndrome, one of acromegaly, one of hyperinsulismus, and one of climacterium. The results do not differ from the normal level, with the exception of the patient with hyperinsulismus.

Achylia gastrica: The material was made up of 7 histamin resistant achylia patients who suffered from no other noteworthy disease. The average value of pyridinenucleotides was normal both in whole blood and in red cells. In case 6, where the red cells value was low, the patient in question was a man of 75, who in addition to achylia suffered from serious heart insufficiency, having among other things, stasis in the lungs, his general condition being rather pool (Table VII).

TABLE VII

PYRIDINENUCLEOTIDES OF BLOOD IN DISEASES OF THE DIGESTIVE SYSTEM

No	Age	Sex	Hematocrit	Pyridinenucleotides		Diagnosis
				In Whole Blood γ per ml.	In Red Cells γ per ml;	
1	20	m	48	38.6	79.6	Achylia gastrica
2	44	»	40	27.6	69.1	» »
3	40	»	40	26.3	65.8	» »
4	61	»	40	21.2	53,0	» »
5	59	f	41	25.7	62.8	» »
6	75	m	46	23.1	50.3	» »
7	50	»	45	24.8	55.2	» »
			Mean 42.9±1.02	Mean 26.8 ±2.04	Mean 62.2 ±3.98	
8	46	m	47	29.1	61.9	Duodenal ulcer
9	29	»	48	28.6	59.6	» »
10	49	»	44	29.4	66.8	» »
11	49	»	48	37.7	78.5	Gastric ulcer
12	79	f	30	22.0	73.2	» »
13	32	m	45	22.9	50.9	» »
14	69	»	36	18.9	52.7	» »
15	49	»	37	22.3	60.3	» »
16	65	»	40	26.4	67.0	» »
17	65	»	35	23.6	67.5	» »
18	21	»	50	32.7	65.4	» »
19	48	»	45	24.4	53.2	» »
			Mean 42 ±1.83	Mean 26.5 ±1.52	Mean 63.1 ±2.41	
20	78	m	30	23.6	78.6	Cholecystopathia
21	45	f	42	21.1	50.3	»
22	36	m	39	20.6	52.8	Hepatitis acuta
23	33	»	48	21.5	46.9	Cirrhosis hepatis
24	56	»	41	23.4	59.5	» »
25	20	f	43	23.0	53.6	Dyspepsia fermentativa
26	26	»	43	23.4	59.5	» »
27	33	m	29	30.3	104.5	Colitis
28	35	f	39	28.1	72.0	»
29	28	»	38	22.9	60.4	»
30	57	»	36	30.6	85.0	Polyposis colonis

TABLE VIII

PYRIDINENUCLEOTIDES OF BLOOD IN LEUKEMIAS AND IN OTHER BLOOD DISEASES

No	Age	Sex	Hemato-crit	Pyridinenucleotides		Diagnosis	
				In Whole Blood γ per ml.	In Red Cells γ per ml.		
1	52	f	29	26.1	90.0	Leucaemia myeloica	Le 42800
2	23	m	27	31.8	117.4	» »	» 75000
3	43	»	41	30.7	74.8	» »	» 70000
4	28	»	17	16.7	98.3	» »	» 13800
5	56	f	41	24.8	60.5	Leucaemia lymphatica	» 19900
6	40	m	45	25.4	56.5	» »	» 34000
7	43	f	27	18.6	68.9	Anaemia perniciosa	Hgb. 45 per cent Er. 1.8 mill. Color Ind. 1.18
8	50	m	42	25.5	60.8	» »	Hgb. 61 per cent Er. 2.7 mill. Color Ind. 1.09
9	52	f	48	43.1	95.8	Polycythemia medicata.	Hgb. 74 per cent Er. 4.7 mill.
10	62	m	65	45.0	69.2	Polycythemia	Hgb. 130 per cent Er. 7.3 mill.
11	52	»	39	26.8	68.7	Myelomatosis	
12	42	f	38	26.8	70.5	Lymphogranulomatosis maligna	

Duodenal and gastric ulcer: This was a group of 12 patients. The diagnosis was established on the basis of a typical case history and X:ray findings. During the examination the patients were on a diet containing mashed potatoes, rolled oats porridge, shred ded meat, fish, milk, gruel, and vegetables. As can be seen from the Table VII, the averages of pyridinenucleotides were normal, which also applies to most individual cases. A more pronounced decrease of pyridine-nucleotides in red cells is to be found only in case 13, in whole blood in case 14. In the former case the patient had suffered from indigestion for 10 years, but the gastric ulcer had been diagnosed one week before the examination. The patient in case 14 had a bleeding ulcus and his general condition was deteriorated.

Hepatic and intestinal diseases: This group was made up of 5 cases of hepatic and 6 of intestinal disease. The Table shows that the pyridinenucleotide values vary considerably, but in the majority of the cases decreased values, however, prevail. The lowest figure,

TABLE IX

PYRIDINENUCLEOTIDES OF BLOOD IN HEART AND CIRCULATORY DISEASES

No	Age	Sex	Hematocrit	Pyridinenucleotides		Diagnosis
				In Whole Blood γ per ml.	In Red Cells γ per ml.	
1	33	m	37	23.1	62.5	Vitium valv. mitralis
2	61	»	38	21.2	55.8	Infarctus cordis
3	19	f	35	23.8	68.0	Endocarditis
4	59	»	25	18.8	75.2	»
5	42	m	45	29.3	65.2	Angina pectoris
6	42	f	44	35.3	80.2	Arteriosclerosis
7	37	m	49	31.2	63.7	»
8	44	»	45	39.8	75.1	»
9	52	f	40	22.3	55.7	»
10	39	m	43	39.8	78.6	Hypertension
11	54	f	35	29.4	85.2	»
12	36	m	43	28.6	66.5	»
13	43	f	44	28.6	64.8	»
14	50	m	45	24.8	52.8	»
15	23	f	34	24.1	70.9	Nephrosis
16	37	m	37	23.8	64.4	Pyelonephritis chr.
17	30	»	44	25.4	53.2	Nephrosclerosis benigna

46.9 γ pel ml., occurred in case 23, where the patient suffered from cirrhosis hepatis and, in consequence, from ascites and hydrothorax, his temperature being 38.5° C. On the other hand, in the second case of cirrhosis (case 24), which was also accompanied with ascites, the general condition was better and the patient afebrile, the figures showing only a slight decrease.

Leucemia and other blood diseases: As is to be seen from the Table VIII, the four cases of leucaemia myeloica that belong to this group show all of them values above normal. In case 2, indeed, we meet the highest figure for pyridinenucleotides in red cells in the entire material, viz. 117 γ per ml. On the other hand, the two patients with leucaemia lymphatica display lower pyridinenucleotide values. As regards the two cases of polycythemia, one of which (case 9) had already repeatedly beem under treatment and at the time of the examination was clinically free from symptoms while the other was being treated for the first time (case 10), it will be found that both have very high pyridinenucleotide values in whole

TABLE X

PYRIDINENUCLEOTIDES OF BLOOD IN RHEUMATIC DISEASES

No	Age	Sex	Hematocrit	Pyridinenucleotides		Diagnosis
				In Whole Blood γ per ml.	In Red Cells γ per ml.	
1	15	m	39	21.9	56.2	Febris rheumatica
2	15	»	43	29.2	67.6	» »
3	37	f	38	30.1	79.8	» »
4	37	»	34	25.8	75.9	» »
5	16	»	34	24.1	70.3	» »
6	15	m	39	30.6	78.4	» »
7	56	f	36	28.6	79.5	Rheumatoid arthritis
8	61	»	38	24.5	64.5	»
9	46	»	36	24.8	68.8	»
10	40	f	42	28.2	67.2	Spondylarthrosis deformans
			Mean 37.9 ± 0.97	Mean 26.8 ± 0.93	Mean 70.9 ± 2.41	

blood and one of the two also in red cells. The high hematocrit of the untreated patient is striking; on account of that the pyridinenucleotide value in red cells does not reach the corresponding high level in whole blood, where it amounts to as much as 45.0 γ per ml.

Heart and circulatory diseases: This group consisted of 14 cases of heart and circulatory diseases as well as of 3 cases of renal diseases. It may be seen from the Table IX that on the whole the pyridinenucleotide values are normal. Decrease is to be seen in case 2, the patient in question suffering from infarctus cordis in acute phase; a few days after the examination the patient died. Also one patient with arteriosclerosis and one with hypertension (cases 9 and 14) had decreased pyridinenucleotide values in blood. The general condition of each patient was somewhat below normal, but there were no symptoms of inflammatory diseases to be found.

As regards the three renal cases, two had normal pyridinenucleotide values, but the third patient, suffering from nephrosclerosis benigna, shows decreased values in red cells.

Rheumatic diseases: The averages and individual values show that in rheumatic diseases the pyridinenucleotide amount in whole blood as well as in red cells is normal. Only in case 1 we meet a lower value. The patient in question, suffering from rheumatic fever

TABLE XI

PYRIDINENUCLEOTIDES OF BLOOD IN CARCINOMA AND SOME OTHER MALIGNANT TUMOURS

No	Age	Sex	Hematocrit	Pyridinenucleotides		Diagnosis
				In Whole Blood γ per ml.	In Red Cells γ per ml.	
1	50	m	41	25.1	61.2	Carcinoma pulmonis
2	62	»	36	23.1	64.2	» »
3	52	»	43	31.8	74.0	» »
4	64	»	34	22.2	65.2	Carcinoma ventriculi
5	72	»	44	25.8	58.6	Carcinoma pancreatis
6	51	»	51	23.8	46.7	» »
7	50	»	41	21.3	52.0	Tumor mediastini
8	28	»	41	23.0	56.2	Seminoma testis

had among other ailments also swollen joints. The other case of rheumatic fever, where the pyridinenucleotide value in blood was normal, had already passed the acutest phase of the disease.

Carcinoma and some other malignant tumours: In the case of both pulmonary and gastric carcinoma the pyridinenucleotide value was normal. The other four patients in this group, however, showed slightly lower values, in case 6 there being a considerably decreased value. The patient in question had carcinoma pancreatis accompanied with rather advanced jaundice, his general condition being poor. The case with seminoma testis was discovered to have developed extensive metastases, which had reached already the lungs.

COMMENTS

In the preceding investigation it has been found out that the total amount of pyridinenucleotides in healthy subjects averages 28.1 ± 0.69 γ per ml. in whole blood and 67.1 ± 1.49 γ per ml. in red cells. These values are in agreement with the figures obtained by earlier researchers by means of the biological or the fluorometric method.

The determination of pyridinenucleotides in blood in a total of 253 cases of illness, of which the largest is made up of 92 cases of tuberculosis, showed that the values are decreased in certain disease

groups, most celarly in tuberculosis, less obviously in schizophrenia and neurocirculatory asthenia. In the other disease groups no values below normal occurred, except in some individual sporadic cases over the entire material (Table XII). Values above normal were found in patients ill with leucemia, evidently as a consequence of increased leukocytes. Some groups were made up of so few cases that no definite conclusions could be drawn. The patients examined for the present work came from different parts of the country and had been eating practically the same kind of food; accordingly these factors cannot have influenced the pyridinenucleotide values obtained by the authors.

The pyridinenucleotides for the entire tuberculosis group were 24.0 ± 0.34 γ per ml. in whole blood and 54.0 ± 0.60 γ per ml. in red cells. By comparing these figures with those in healthy persons we find that in terms of statistical mathematics the decrease is almost significant in whole blood and highly significant in red cells. As a rule, the determination was performed before actual therapeutic measures were begun. The amount of pyridinenucleotides did not decisively depend on the stage of the disease, but rather on the nature of tuberoulosis. The most pronounced decrease of values was to be found in the exudative type of the disease, particularly when accompanied with cavities in the lungs. In such cases the average of pyridinenucleotides was 22.9 ± 0.58 γ per ml. in whole blood and 49.2 ± 1.26 γ per ml. in red cells.

Clearly reduced values were also met with in a few cases of pneumonia. This is in accordance with the earlier investigation by Vilter and others.

As regards diseases of the nervous system, clearly decreased pyridinenucleotide values occurred in schizophrenia, the average being 24.6 ± 0.72 γ per ml. in whole blood and 56.2 ± 1.28 γ per ml. in red cells from the viewpoint of statistical mathematics the decrease is almost significant. In neurocirculatory asthenia (neurasthenia, effort syndrome, anxiety neurosis) the corresponding values are 25.4 ± 0.87 and 59.0 ± 1.69, the difference being in this case almost significant.

Normal pyridinenucleotide amounts in blood were established in the following diseases or disease groups: bronchial asthma, rheumatic diseases, gastric and duodenal ulcer, achylia gastrica, diabetes mellitus, and adiposity, either alone or accompanied with

TABLE XII

	Total number of cases	Number of cases with pyridine-nucleotide ranging in red cells below 50 γ per ml.	Number of cases with pyridine-nucleotide ranging in red cells between 50—60 γ per ml	Number of cases with pyridine-nucleotide ranging in red cells between 60—70 γ per ml	Number of cases with pyridine-nucleotide ranging in red cells higher than 70 γ per ml.
Normal series	41	0	12	13	16
Tuberculosis pulmonum..	79	17	52	10	0
Other tuberculosis	6	0	2	4	0
Pleuritis exsudativa	7	1	5	1	0
Asthma bronchiale	7	0	2	3	2
Bronchopneumonia	4	0	4	0	0
Other lung diseases	6	0	2	3	1
Neurocirculatory asthenia	25	3	13	7	2
Schizophrenia	13	1	8	4	0
Other psychiatric and nervous diseases	4	0	4	0	0
Diabetes mellitus	6	0	3	0	3
Struma non toxica and thyreotoxicosis	10	0	5	5	0
Other endocrine diseases..	5	0	1	1	3
Adiposity	6	0	3	1	2
Achylia gastrica	7	0	3	3	1
Duodenal and gastric ulcer	12	0	4	6	2
Hepatic diseases	5	1	3	0	1
Diseases of intestini	6	0	2	1	3
Leucemia and other blood diseases	12	0	1	5	6
Rheumatic and bone diseases	10	0	1	4	5
Heart and circulatory diseases	12	0	1	6	5
Renal diseases	3	0	1	1	1
Carcinoma and other malignant tumours	8	1	3	3	1

hypertension. The distribution of pyridinenucleotide values in the different diseases is to be seen in Table XII.

The method employed by the present authors barely gives the total amount of pyridinenucleotides; thus the different coenzymes or their oxidized or reduced form cannot be specified. The pyridinenucleotide content of blood may be said to give a good general

picture of the active nicotinamide metabolism of the organism, but naturally it doses not reflect the distribution of the vitamins between the different organs. The pyridinenucleotide content of blood gives us a general idea of the nicotinamide economy of the body. The results of the present study show that in healthy individuals the pyridinenucleotides in blood seldom fall far below the normal average. Low pyridinenucleotide content in blood is characteristic of certain disease groups and occurs only occasionally in the entire material.

It seems permissible to conclude that there is no noteworthy nicotinamide deficiency in this country. Typical diseases caused by nicotinamid deficiency are on the whole rare in Finland.

But what, then, is the explanation of the low pyridinenucleotide values in blood that were met with in certain diseases and disease groups? As has been pointed out above, in some cases this may be due to the poor general condition of the patient. A survey of the disease groups in which the lowest values turned up, renders it apparent that the persons that these are made up of would be classified according to the present way of thinking as belonging to the leptosome or the athletic type of constitution. Persons ill with schizophrenia are also regarded as having a greater than normal disposition to contract tuberculosis. In view of this it is difficult to dismiss from one's mind the thought that the low pyridinenucleotide values in blood in such disease groups as schizophrenia and neurocirculatory asthenia are not caused by the patient's illness but would reflect his inborn disposition to certain metabolic disturbance.

With reference to points of view put forward above it does not seem impossible to apply this explanation also to tuberculosis. Previous investigations from our Department on the occurrance of cocarboxylase, the coenzyme form of vitamin B_1 in blood in various diseases and disease groups (Kerppola and Vartio 1953, Vartio 1953) showed that decreased values are to be found in only one disease group, viz. neurocirculatory asthenia. In schizophrenia and tuberculosis the values were normal. Accordingly it has been found out that in neurocirculatory asthenia there is disturbance in two different kind of vitamin B.

As has been explained above, pyridinenucleotides act as coenzymes of many enzymes, particularly of dehydrogenases. If there

are absent, the metabolism of both carbohydrates and fat as well as proteins is changed. This tends to cause disturbance in cell function and probably also to lower the subject's power of resistance against infection. It is a well-known fact that the tubercle bacillus very much depends on pyridinenucleotides as regards its metabolism; the use of isoniazid an inactive nicotinamide form, in the treatment of tuberculosis is probably based on this state of affairs (Pätiälä 1954).

SUMMARY

Pyridinenucleotides in blood have been determined by the fluorometric method of Nora Levitas and others in 41 healthy subjects and in 253 different cases of illness. The largest group, one of 92 cases, is made up of tuberculous patients with 25 cases of neurocirculatory asthenia and 17 cases of schizophrenia coming next. In all these groups lower than normal values of pyridinenucleotides were found in blood.

Diagnosis	Pyridinenucleotides	
	In Whole Blood γ per ml.	In Red Cells γ per ml.
Tuberculosis	24.0 ± 0.34	54.0 ± 0.60
Schizophrenia	24.6 ± 0.72	56.2 ± 2.80
Neurocirculatory asthenia	25.4 ± 0.87	59.0 ± 1.69
Normal cases	28.1 ± 0.69	67.1 ± 1.49

In the other disease groups no values below normal occurred except in some individual cases here and there in the whole material. Values higher than normal were met with in patients with leucemia.

The amount of pyridinenucleotides in tuberculous patients was not decisively dependent on the stage of the disease but on the nature of tuberculosis. The most highly decreased values were found in the exudative type of the disease, particularly in cases with cavities in the lungs.

When looking for an explanation of the decreased pyridinenucleotide values in blood found out in the above-mentioned disease

groups it does not seem possible to the authors to look upon them as simply a consequence of the disease the subjects in question were suffering from, but rather as a reflection of a certain inborn disposition of these persons to the metabolic disturbance described above.

REFERENCES

Bezancon, J.: Cit. Rossini et Cattaneo.

Elvehjem, C. A., Madden, R. J., Strong, F. M., and Woodley, D. W.: The Isolation and Identification of the Anti-Black Tongue Factor. J. Biol. Chem. 1938:*123:*137.

Forsman, V., and Larmola, E.: Keuhkotuberkuloosin kliininen jako. Suomen Lääkärilehti (Finnish). 1944:*3:*115.

Jokipii, S. G.: Experimental Studies on Blood Alcohol in Healthy Subjects and in Some Diseases. An. med. experim. et biol. Fenniae. 1951:*29:* Suppl. 2.

Kerppola, W., and Vartio, T.: Cocarboxylase Content of Human Blood Studies with Normal Subjects and in Neurocirculatory Asthenia, Thyreotoxicosis, Adiposity and Somer Other Diseases. A Preliminary Report. An. med. experim. et biol. Fenniae. 1953:*31:*111.

Kohn, H. J.: The Concentration of Coencyme like Substances in Blood Following the Administration of Nicotinic Acid to Normal Individuals and Pellegrins. Biochem. J. 1938:*32:*2075.

Levitas, N., Robinson, J., Rosen, F., Huff, J. W., and Perlzweig, W. A.: A Rapid Fluorometric Method for the Determination of the Total Pyridinenucleotides in the Red Blood Cells. J. Biol. Chem. 1947:*167:*169.

Oliva et Magrini: Cit. Rossini et Cattaneo.

Pätiälä, J.: The Amount of Pyridinenucleotides (Coenzymes I and II) in Blood in Experimental Tuberculosis Before and During Isonicotinic Acid Hydrazide Treatment. Am. Rev. Tuberc. 1954:*70:*453.

Rossini, G.: Valori di codeidrasi nei soggetti tubercolotici. Acta vit. (It.). 1949:*6:*243.

Rossini, G. et Cattaneo, P.: Sul comportamento del fattore PP nel corso delle affecioni tubercolari. Minerva med. (It.). 1951:*42:*149.

Spies, T. D.: Pellagra. Cecil's Textbook of Medicine, ed. 4, Philadelphia, W. B. Saunders Co., 1937.

Sydenstricher, V. P., Geeslin, L. E., Weaver, G. W.: Avitaminosis Occuring in Diabetic Patients Under Insulin Therapy. J.A.M.A. 1939: *113:*2137.

Vartio, T.: The Cocarboxylase Content of Blood in Normal Subjects and in Various Diseases, Expecially Neurocirculatory Asthenia. Scandinav. J. Clin. Lab. Investigation 1953:*5:*Suppl. 10.

VILTER, R. W., VILTER, S. P., and SPIES, T. D.: Relationship Between Nicotinic Acid and a Codehydrogenase (Cozymase) in Blood of Pellagrins and Normal Persons. J.A.M.A. 1939:*112:*420.

VILTER, R. W., POTTER, S., and SPIES, T. D.: Determination of the Codehydrogenases I and II (Cozymase) in the Blood of Diabetics in Severe Acidosis. Am. J. M. Sc. 1939:*197:*323.

VILTER, R. W., BEAN, W. B., RUEGSEGGER, J. M., and SPIES, T. D.: The Role of Coenzymes I and II in Blood of Persons with Pneumococcal Pneumonia. J. Lab. & Clin. Med. 1940:*25:*897.

VILTER, S. P., KOCH, M. B., and SPIES, T. D.: Coenzymes I and II in Human Blood. J. Lab. & Clin. Med. 1940:*26:*31.